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STUDIES IN MENINGOCOCCUS INFECTIONS.*

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INTRODUCTION.

A NUMBER of cases of epidemic cerebrospinal meningitis appeared at Cook County Hospital during the spring of 1905. A study of these was begun with particular reference to the bacteriological flora of the nose and throat with a view of determining the frequency of the presence of hemophilic bacteria, these organisms having been studied in several other infections during the winter. To do this complete bacteriological examinations were made of the secretions of the nose and throat upon blood-agar plates and a record made not only of the hemophilic bacteria but also of the various other organisms which appeared on the plates and in smears of the secretions. A bacteriological study was made at the same time of the cerebrospinal fluid and also in all cases but one of the blood. A rather extensive study of the meningococcidal properties of blood and serum of normal persons and of patients with meningitis was also made.

The cases all occurred in Italians recently arrived from Italy. Two of the cases were taken sick on the train coming from New York where they had obtained quarantine papers only a few days previously. This is interesting in view of the fact that an epidemic of this disease was raging in that city at the time. There is no evidence, however, to indicate that these patients were in any way exposed directly to the disease at that place.

A brief report of the individual cases is given below.

CASES.

Case 1.—Italian. Age 19. Taken sick on train from New York. He had a typical attack of meningitis of the fulminant type, dying in less than 24 hours after entering the hospital. The cerebrospinal fluid obtained by spinal puncture was turbid and showed almost exclusively polymorphonuclear leucocytes, many of which contained Gram-negative diplococci. The organisms

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were also numerous outside the leucocytes. Cultures showed pure growths of the meningococcus (Diplococcus intracellularis, Weichselbaum). There was considerable expectoration and the sputum was purulent. Smears of the sputum showed many pus cells. The predominating organism was a small Gram-negative bacillus resembling the influenza bacillus. Gram-negative diplococci were numerous. A few Gram-positive diplococci (pneumococci) were present and also some Gram-negative diplobacilli. The pus cells contained some Gram-negative diplococci. Cultures of the sputum showed numerous colonies of hemophilic bacilli, a number of colonies of meningococci, a few colonies of pneumococci, and a few colonies of Gram-positive cocci. Smears of the nasal secretion showed many Gram-positive bacilli resembling pseudo-diphtheria, some Gram-negative biscuit-shaped diplococci, and a considerable number of small Gram-negative bacilli. Cultures gave numerous colonies of hemophilous bacilli, many colonies of meningococci, and some Gram-positive bacilli, probably pseudo-diphtheria. The above material was obtained 12 hours before death. Unfortunately, no blood cultures were obtained in this case.

Case 2.—Italian. Age 19. Taken sick on train from New York. Typical case of acute meningitis, but less severe than Case 1. Cerebrospinal fluid was turbid and contained many polymorphonuclear leucocytes with numerous Gram-negative diplococci within them. The same organisms were also numerous outside of leucocytes. The meningococcus was obtained in pure culture from the fluid. Smears of the sputum showed Gram-positive diplococci, some Gram-positive bacilli resembling diphtheria bacillus and a few large Gram-negative bacilli. Cultures gave numerous colonies of pneumococci, a few of streptococci, none of hemophilic bacilli or meningococci. Smears of the nasal secretion showed a number of Gram-negative organisms suggestive of meningococcus, and a few Gram-positive cocci. A second examination a few days later showed small, Gram-negative bacilli suggestive of influenza bacilli in abundance. Cultures gave, in both examinations, hemophilous bacilli in large numbers, a few pneumococci, and some hemolytic colonies of a Gram-positive coccus (staphylococcus). No meningococcus colonies were obtained on the plates. Blood cultures at three different times remained sterile. This case recovered after several weeks' sickness.

Case 3.—Italian. Adult. Typical case of epidemic meningitis. The meningococcus was obtained in smear and culture from the cerebrospinal fluid. Smears of the nasal secretion gave a few Gram-negative, biscuit-shaped diplococci and numerous chains and pairs of Gram-positive cocci (streptococci). The cultures showed many pneumococcus and streptococcus colonies, and a number of colonies of hemophilous bacilli. No colonies of meningococcus or Micrococcus catarrhalis were obtained in the cultures. The patient died after a few days' illness and a post-mortem was obtained. The meningococcus was isolated from the exudate about the brain and spinal cord. A purulent pericarditis was present, but the exudate showed no organisms in smear or culture. The nasal sinuses contained a large amount of a dirty, greenish mucus which revealed numerous streptococci, and in smear a considerable number of Gram-negative diplococci, but they did not grow on blood-agar plates. The heart's blood was sterile.

Case 4.—Italian. Age 16. Sick four days before entering the hospital. Typical case of meningitis. Recovery after several weeks' sickness. The meningococcus was isolated in pure culture from the cerebrospinal fluid. Smears of the fluid showed the diplococci in abundance. Blood culture gave a negative result. Smears of the nasal secretions (seventh day of disease) showed a large number of pseudo-diphtheria bacilli, many Gram-positive cocci and a few Gram-negative diplococci resembling the meningococcus. Cultures showed a large number of staphylococcus and streptococcus colonies, many pseudo-diphtheria colonies, but no meningococcus colonies or hemophilous bacilli. Three days later a second examination showed nearly pure growth, both in smear and culture, of hemophilous bacilli. No colonies of meningococcus or Micrococcus catarrhalis were obtained. Sputum could not be obtained for examination.

Case 5.—Italian. Age 26. Sick three weeks. Not a severe case. The cerebrospinal fluid had a greenish tinge, was distinctly turbid and contained numerous polymorphonuclear leucocytes, large endothelial cells and a few mononuclears. No organism could be detected in smears of the fluid. In culture the meningococcus was obtained in pure growth. Blood cultures gave a negative result. The patient showed no nasal or throat symptoms. Smears of a nasal swab showed only Gram-positive cocci and cultures showed numerous colonies of Staphylococcus albus. No other organisms were obtained. Examination of throat swabs showed some pneumococci and a few other Gram-positive cocci.

SUMMARY OF BACTERIOLOGICAL FINDINGS.

In the five cases, therefore, the meningococcus was obtained from the spinal fluid in every instance by culture, and observed in smear in all but one. It was isolated from the nasal cavities and the sputum in one case, and in four of the five cases Gram-negative diplococci suggestive of either meningococcus or Micrococcus catarrhalis were seen in smears but not recovered in culture. The blood in the four cases examined gave negative results.

The meningococci from the different cases were identical in every detail. They were Gram-negative, usually in pairs, often single, frequently in tetrads, and never in chains. They grew well in ascites broth and in blood serum and blood agar, but very poorly or not at all in plain broth. No appreciable growth occurred at room temperature. From these characteristics they evidently belong to the Weichselbaum type of meningococcus and not the Jäger-Heubner type. They were all carried through many generations upon various media, three of them being transferred almost daily on blood serum for several months, without

noting any change in their properties, except perhaps a slightly more vigorous growth.

The close resemblance of the meningococcus to the Micrococcus catarrhalis and the gonococcus has been indicated by a number of observers, particularly by Ghon and Pfeiffer,[1] and Libman and Celler.[2]

The Micrococcus catarrhalis is a common organism of the respiratory passages undoubtedly existing frequently as a saprophyte, though often associated with various kinds of inflammatory conditions of this region, particularly in children. It must always be kept in mind in examining nasal and pharyngeal excretions for meningococcus because of the danger of confusing the two organisms. It might, therefore, not be out of place here to indicate briefly some of their distinguishing characteristics. In smears it is practically impossible to distinguish between them. The Micrococcus catarrhalis in the nasal passages and throat is frequently found inside the leucocytes; so far it has never been found in the cerebrospinal fluid. On the whole the Micrococcus catarrhalis is slightly larger and does not vary in size and form to the extent that the meningococcus does; but these are only uncertain criteria for distinguishing them. Culturally certain differences appear quite distinctly. The meningococcus colonies are slightly yellow, have a clear, homogeneous outer zone with a non-granular or very finely granular central portion. The margin is even. The Micrococcus catarrhalis colonies are much more coarsely granular, more opaque and yellowish-brown, while the margin is gnawed. It grows much more abundantly at room temperature than does the meningococcus, at least the Weichselbaum type, and also much better in broth. The two organisms may vary considerably in their pathogenicity for animals, as will be shown later, and this may be of some aid in distinguishing them, but the most positive method of differentiation is the agglutination test with immune serum. The possibility of intermediate forms should be kept in mind, as the biological characteristics of various diplococci frequently found closely resembling these organisms have not yet been given careful study.

[1] *Ztschr. f. klin. Med.*, 1902, 44, p. 274. [2] *Mt. Sinai Hospital Reports*, 1903, 3, p. 542.

The gonococcus, while practically identical with these organisms morphologically, is easily distinguished culturally because of the difficulty of growing it on ordinary media. The gonococcus has not been found in the nasal passages or in the throat, but in bacteriologic examinations of the eye, blood, and joints and even cerebrospinal fluids for meningococci, this organism should be kept in mind, for it may be found in these places.*

The meningococcus obtained from the sputum and nasal secretion in Case 1 was subjected to the most careful tests to distinguish it from the Micrococcus catarrhalis. Culturally it conformed in every detail to the organism isolated from the cerebrospinal fluid of the same case and gave an agglutination reaction with the serum of Case 5 at a dilution of 1:50. A typical Micrococcus catarrhalis obtained from the throat of a case of measles was used as a control. It was not agglutinated by the serum of Case 5.

While it is a very prevalent idea that the meningococcus occurs commonly in the nasal cavities of meningitis patients and even of those not suffering from the disease, examination of the data upon which this notion is based reveals a surprisingly small amount of reliable evidence in its favor. This is due to the imperfect examinations made of the secretions; for the finding of Gram-negative diplococci in smears and on superficial cultural examination has been frequently considered as sufficient to report the presence of meningococci. The reports of many of the cases in the literature are of this kind and hence of little or no value. Councilman[1] recently quoted Lord as stating that there are only four undoubted cases on record where the meningococcus has been isolated from the nasal cavities. This does not mean necessarily that the meningococcus is rarely present in the nasal and pharyngeal secretions, but merely that most of the present data are worthless so far as definitely demonstrating that fact. It is interesting

*It is scarcely necessary to say anything about differentiation of the pneumococcus from the meningococcus, at least the Weichselbaum type, for the two organisms have so little in common, especially culturally, that there can be little difficulty in identifying them. The absence of any zone of hemolysis about meningococcus colonies on human blood-agar plates and the characteristic zone about the pneumococcus colonies serves as a rapid and convenient means of differentiation.

[1] *Jour. Am. Med. Assn.*, 1905, 44, p. 999.

that recent evidence derived from careful cultural and agglutinative tests appears to confirm an older notion founded on absolutely unreliable data. The question as to the frequency of the presence of the meningococcus in the nasal cavities and smears of epidemic meningitis patients and the relation it bears to the frequent complicating rhinitis is therefore, as Weichselbaum says,[1] still an open one.

A number of animal experiments were made with the meningococcus isolated from our cases. On the whole they show rather feeble pathogenicity. When inoculated in sufficient quantity intraperitoneally into guinea-pigs, the animal dies within 24 hours of an acute peritonitis. The exudate contains immense numbers of leucocytes, all of which are packed full of the organisms. Three guinea-pigs of the same size were selected and inoculated intraperitoneally: one with the organism from the cerebrospinal fluid of Case 1, the second with the organism isolated from the nasal secretion of the same case, and the third animal with the same quantity of a culture of Micrococcus catarrhalis isolated from a case of measles. The first two died in 18 hours and the meningococcus was obtained from the pleural fluid and the heart's blood of each. The third animal showed no symptoms whatever. As stated above, this may be used as a means of differentiating the two organisms, but it is known according to Kirchner[2] and to Neisser[3] that occasionally the Micrococcus catarrhalis is feebly pathogenic also, so that such results would not always be reliable.

Attempts were made to inoculate monkeys by swabbing the nasal mucosa and the throat with cultures and also by forcing the material by sprays and swabs high up into the nasal cavities in the region of the cribriform plate. The results were all negative, not the slightest evidence of any symptoms appearing. Two slant cultures from blood serum were also injected directly into the vein of a small monkey without having the least effect.

It is of interest that hemophilous bacilli were obtained in four of the five cases, and that in two of these they were decidedly the

[1] KOLLE AND WASSERMANN, *Handb. der path. Mikroorg.*, 1903, 3, p. 277.

[2] *Ztschr. f. Hyg.*, 1890, 9, p. 528.

[3] KOLLE AND WASSERMANN, *ibid.*, p. 146.

predominating organism. These organisms only grew on hemoglobin media, and morphologically and culturally in every way corresponded to the influenza bacillus. Those from Case 2 and Case 4 had a distinct tendency to form rather long threads. These cases did not show especially marked coryzal symptoms except Case 3, in which there was an abundant secretion of a viscid mucus. There was, however, considerable increase in mucus, which accumulated far back in the nasal cavities and could be obtained on the swab. From what is known of this influenza-like organism it would not be unreasonable to attribute to it such symptoms as the above. A study of nasal secretions of cases of meningitis with especial reference to these organisms should be made, and it may be found that they are concerned in some way with the coryzal symptoms often observed in meningitis.*

Pseudo-diphtheria organisms were obtained in two cases. These are similar to the organisms of this character found frequently on the normal nasal mucosa, and are probably of little significance. The streptococcus was obtained from the nasal cavity in Case 3 in large numbers and in small numbers from the sputum of the same case. These organisms produced a wide, clear zone of hemolysis on the blood-agar plates and occurred in long chains. Gram-positive bacilli resembling staphylococci were commonly found in the nasal secretions, but are probably of little significance, being of the same character as those found so frequently normally.

AGGLUTINATION.

Agglutination tests were made in four cases by the microscopic method. The organism from Case 5 was agglutinated by the patient's serum in dilution of 1:100 in 30 minutes. It was practically complete in one hour. With two normal sera the organism showed no indication of clumping even in pure serum or diluted one-half. The serum from Case 4 agglutinated the organism from Case 2 in a dilution of 1:50 in 30 minutes, and the same organism was agglutinated by its homologous serum in

*It is to be noted that at the time these cases of meningitis appeared there were no cases of influenza in the city to speak of, and hence the presence of these organisms cannot be attributed to a prevalent influenza infection.

the same dilution. Controls with normal serum showed no agglutination whatever.

An opportunity was offered to test the serum of a young man who had had a typical attack of meningitis, from whose cerebrospinal fluid the meningococcus was obtained two years and four months previously. There was distinct agglutination at a dilution of 1:2 and only very slight clumping after one hour at 1:10. Above this no effect was noted.

The data are insufficient to decide as to the time of the appearance of the reaction, but at the ninth day, as shown in Table 1, the reaction is well marked.

TABLE 1.

AGGLUTINATION OF MENINGOCOCCUS.

CASE	TIME AFTER ONSET OF DISEASE	DILUTION				SOURCE OF ORGANISM
		1:10	1:20	1:50	1:100	
2.....	4th week	+	+	+	0	Case 2
4.....	9th day	+	+	+	0	Case 2
5.....	3d week	+	+	+	+	Case 5
6.....	2 yrs. 4 mo.	+	0	0	0	Case 5
Normal serum I..	.....	0	0	0	0	Case 2
Normal serum II.	.....	0	0	0	0	Case 5

The agglutination test may be of great help in the diagnosis of these cases, particularly those in which cultures of spinal fluid give negative results, as not infrequently occurs. In Case 5 in this series the first cultures made from the spinal fluid were sterile, and there was considerable doubt as to the diagnosis in view of the fact that he had been sick over three weeks and was running a chronic, mild course. In a second attempt no organisms could be seen in smears of the fluid, and only by the most careful technique were a few colonies finally obtained in culture. The agglutinative reaction, however, was clearly obtained at a dilution of 1:100 in 30 minutes. In suspicious cases, should no organism be found in the cerebrospinal fluid, it is therefore advisable to try the agglutination test. It is valuable also in distinguishing the epidemic form of meningitis caused either by the Weichselbaum or the Jäger-Heubner type of meningo-

coccus from meningitis caused by other organisms, as the pneumococcus, streptococcus, tubercle bacillus, etc. For Sorgente[1] and others have shown by extensive animal experiments that both types of meningococcus react alike to immune serum, while various other diplococci from different sources and closely resembling the meningococcus are not agglutinated by the serum of animals immunized to the meningococcus.

EFFECT OF DEFIBRINATED BLOOD, BLOOD SERUM, AND CEREBRO-SPINAL FLUID ON THE MENINGOCOCCUS.

Defibrinated blood.—Experiments were made to determine the bactericidal effect of normal blood and blood from patients suffering from meningitis, and also of other fluids upon the meningococcus. The method used consisted in introducing into small

TABLE 2.

EFFECT OF NORMAL BLOOD ON MENINGOCOCCUS.

	At Once	3 Hours	6 Hours	20 Hours	30 Hours
1. Normal Blood I .6 c.c.....	17	71	38,400	∞	....
2. " " " "	112	0	1,952	∞	....
3. " " " "	352	24	192	∞	....
4. " " " "	1,208	3	0	0	....
5. " " " "	2,800	10	150	∞	....
6. " " " "	3,300	6	0	232	....
7. " " II "	960	3	1	10,000	....
8. " " " "	1,500	50	320	∞	....
9. " " " "	1,900	350	3,000	∞	....
10. " " III "	856	5	0	0	....
11. " " IV "	3,120	0	0	0	....
12. " " V "	1,184	0	0	0	....
13. " " " "	20,000	176	55	7,200	∞
14. " " VI "	552	0	0	0	....
15. Dog's Blood "	880	0	0	0	....

NOTE.— ∞ indicates over 15,000.

tubes accurately measured quantities of the material to be tested (blood, serum, spinal fluid, etc.) and this was inoculated with a uniform quantity of a 24 hour culture of meningococcus. Ascites-agar plates (1 part heated ascites fluid to 4 parts plain agar) inoculated with a loop from the tubes were made at once and at varying intervals of time thereafter, usually 3, 6 and 18 or 24 hours. The colonies appearing upon the plates were counted after 48 hours.

The bactericidal property of defibrinated blood from six normal adults was tested on the meningococcus. The results are

[1] *Centralbl. f. Bakt.*, 1905, 39, p. 1.

not uniform, there being considerable individual variation. In blood from two of the six cases the organisms grew abundantly. One of these was tested at six different times and in every instance but one, good growth occurred. In the other four cases no colonies appeared on the plates after three hours. Table 2 shows the number of colonies on the plates at various periods. It is to be noted that in nearly every instance a rapid decrease occurs in the first few hours. This may be due to phagocytosis, which will be discussed in greater detail later on.

The individual variation in the blood from normal persons is interesting in view of the possible relation it may bear to susceptibility to infection.

Blood from three cases of meningitis was tested, and in every instance no growth occurred in the plates after three hours (Table 3). Unfortunately blood was not obtained from any of

TABLE 3.

EFFECT OF MENINGITIC BLOOD ON MENINGOCOCCUS.

		Time After Onset	At Once	3 Hours	6 Hours	20 Hours
1. Men. Blood (Case 2)	.6 c.c.....	5th week	16	0	0	0
2. " " "	.6 c.c.....	6th week	90	0	0	0
3. " " "	.6 c.c.....	6th week	536	7	0	0
4. " " "	.6 c.c.....	7th week	282	1	0	0
5. " " (Case 4)	.6 c.c.....	10th day	40	0	0	0
6. " " "	.6 c.c.....	13th day	1,296	0	0	0
7. " " (Case 5)	.6 c.c.....	3d week	1,400	0	0	0

these cases earlier than the tenth day. It would be very desirable to test the blood at the time of the onset and later in the disease; also to test the blood in fatal cases, for as it happened each of the three cases tested recovered. This also applies to the experiments with meningitic sera given below.

Normal and meningitic sera.—Differing from the streptococcus and pneumococcus, the meningococcus does not grow in normal or meningitic sera, being quickly killed in from one to two hours. Neither will it grow in heated sera (60–65° C. for 30 minutes), though in this the bactericidal power is somewhat diminished. In order to determine this power, varying quantities of the serum were added to ascites broth, in which it grows well, and plates made as usual. The result is shown in Table 4.

Experiments were repeated with a number of normal sera and also with sera from three cases of meningitis with uniform results. The data given in this table are not composite but are from a series made at one time with the same organism, the same normal and immune blood, and the same ascites broth. They represent very typically the results that were obtained with various other sera and other strains of organisms. In this table also is given the effect of heated serum added to washed corpuscles, which it was found furnishes a most excellent medium for this organism.

TABLE 4.

EFFECT OF SERA ON GROWTH OF MENINGOCOCCUS.

		0 Hrs.	3 Hrs.	6 Hrs.	18 Hrs.
1.	Normal Serum .6 c.c.	1,150	0	0	0
2.	" " .4 c.c. + Asc. Broth .2 c.c.	1,280	80	75	0
3.	" " .3 c.c. + " " .3 c.c.	1,800	85	50	560
4.	" " .2 c.c. + " " .4 c.c.	42	336	1,600	∞
5.	" " .1 c.c. + " " .5 c.c.	640	660	10,000	7,000
6.	Normal Serum heated .6 c.c.	1,000	10	0	0
7.	" " " .4 c.c. + Asc. Broth .2 c.c.	1,150	960	820	240
8.	" " " .3 c.c. + " " .3 c.c.	1,200	950	2,200	∞
9.	" " " .2 c.c. + " " .4 c.c.	1,150	1,100	1,800	∞
10.	" " " .1 c.c. + " " .5 c.c.	1,300	1,500	∞	∞
11.	Meningitis Serum (Case 5) .6 c.c.	960	1	0	0
12.	" " " .4 c.c. + Asc. B. .2 c.c.	800	37	12	0
13.	" " " .3 c.c. + " " .3 c.c.	640	90	69	2
14.	" " " .2 c.c. + " " .4 c.c.	672	360	104	27
15.	" " " .1 c.c. + " " .5 c.c.	1,120	960	92	216
16.	Men. Serum heated .6 c.c.	650	0	0	0
17.	" " " .4 c.c. + Asc. Broth .2 c.c.	760	150	2	0
18.	" " " .3 c.c. + " " .3 c.c.	1,100	450	380	6
19.	" " " .2 c.c. + " " .4 c.c.	480	520	460	∞
20.	" " " .1 c.c. + " " .5 c.c.	450	490	1,100	∞
21.	Normal Blood .6 c.c.	1,500	50	320	∞
22.	Normal Washed Corp. + Heated Nor. Ser. = .6 c.c.	2,600	2,800	∞	∞
23.	" " " + Men. Serum = .6 c.c.	900	0	0	84
24.	Men. Blood .6 c.c.	1,440	0	0	0
25.	Men. Washed Corp. + Heated Men. Ser. = .6 c.c.	1,650	1,200	650	10,000
26.	" " " + Nor. Ser = .6 c.c.	1,100	0	0	0
27.	Ascites Broth (1:4) .6 c.c. (Control)	1,500	1,550	15,000	∞

The table shows that the bactericidal effect of normal heated serum is not as great as that of normal unheated serum. This is indicated in two ways. If No. 3 (Table 4) is compared with No. 8, in which equal parts of the heated and unheated sera and ascites broth are used, it is seen that the unheated serum strongly inhibits the development of the organisms while No. 8 is an excellent medium for them. Again by comparing Nos. 21 and 22, the latter, which contains the heated serum, is much more favorable for their growth. This was brought out in a still more striking way with the normal bloods referred to in Table 2, in which the meningo-

coccus did not develop. In every such instance heated normal serum added to the washed corpuscles furnished a most excellent medium for their growth. We may therefore say that the bactericidal power of normal serum is very materially diminished by heating to 60° for 30 minutes.

Comparing normal serum with meningitic serum (three weeks after onset) we see that the bactericidal effect of the latter is greater than the former, and we also note from the table that heating the meningitic serum markedly decreases its inhibitory effect, so that there is little difference between heated normal serum and heated meningitis serum. This is also shown very clearly in Nos. 24 and 25. When the serum of meningitic blood is replaced by the same heated serum the organisms grow very well. Here, however, the question of phagocytosis is also involved, as will be explained farther on. When meningitic serum is added to normal washed corpuscles (No. 23) growth does not occur. This result was obtained with three different meningitic sera. When normal serum is added to meningitic washed corpuscles (No. 26) as a rule no growth occurs, though results of several experiments were not uniform, varying in fact much as the results for normal blood varied.

From the above facts, therefore, we may conclude that the meningococcidal power of meningitic serum is greater than that of normal serum and that this property is diminished by heating to 60° for 30 minutes.

Cerebrospinal fluid.—The bactericidal power of cerebrospinal fluid was also determined in the same manner. Table 5 gives the results obtained and also a control with normal salt solution. The cerebrospinal fluid used was from a case of chronic hydrocephalus; it was perfectly clear and contained no cellular elements to speak of. A second experiment with fluid from a case of uremia gave virtually the same result. No growth occurs in the pure fluid, and only when mixed in about equal proportions with ascites broth does the meningococcus thrive. Heating to 60° for 30 minutes does not change its properties essentially. Compared with normal salt solution it is distinctly more unfavorable for their growth. In fact it acts much like normal heated serum.

TABLE 5.

EFFECT OF CEREBROSPINAL FLUID ON THE MENINGOCOCCUS.

		0 Hrs.	3 Hrs.	6 Hrs.	20 Hrs.
1.	Cerebrospinal Fluid .6 c.c.	1,750	51	2	0
2.	" " .5 c.c. + Asc. Broth .1 c.c.	1,300	1,450	3	0
3.	" " .4 c.c. + " " .2 c.c.	1,456	1,700	640	0
4.	" " .3 c.c. + " " .3 c.c.	1,080	1,300	1,400	4,500
5.	" " .2 c.c. + " " .4 c.c.	1,600	1,600	2,200	8,000
6.	" " .1 c.c. + " " .5 c.c.	1,360	1,500	1,950	∞
7.	Cerebrosp. Fl. heated .6 c.c.	2,200	12	0	0
8.	" " " .5 c.c. + Asc. Broth .1 c.c.	2,200	2,350	1,100	0
9.	" " " .4 c.c. + " " .2 c.c.	2,450	2,500	3,500	0
10.	" " " .3 c.c. + " " .3 c.c.	2,800	3,500	3,400	6,000
11.	" " " .2 c.c. + " " .4 c.c.	2,080	1,950	4,200	∞
12.	" " " .1 c.c. + " " .5 c.c.	1,950	2,100	2,800	∞
13.	Ascites Broth .6 c.c. (Control)	1,500	980	1,600	∞
14.	Normal Salt .6 c.c.	4,400	0	0	0
15.	" " .4 c.c. + Ascites Broth .2 c.c.	1,456	1,072	6,000	∞
16.	" " .3 c.c. + " " .3 c.c.	2,880	3,500	5,000	∞
17.	" " .2 c.c. + " " .4 c.c.	3,040	4,000	3,500	∞
18.	" " .1 c.c. + " " .5 c.c.	3,200	3,500	4,200	∞

PHAGOCYTOSIS.

Beside the bactericidal properties of plasma and serum-another factor of importance in explaining the destructive action of blood upon bacteria is phagocytosis. Through the new contributions made to this subject by Wright and Douglas, Hektoen and Ruediger, and others, the details of the mechanisms at work certainly are much clearer and better understood than formerly.

From this point of view a few experiments were made with the meningococcus in order to detect possible differences in the opsonic power of normal blood and meningitic blood. The results are given in Table 6. The method used is that employed by Wright and Douglas, and, briefly, consists in mixing in small tubes definite amounts of the fluids used with bacterial suspension in NaCl solution, incubating at 37° C. for about 10 minutes. Then smears are made and stained with Leishmann's stain and the number of organisms taken up by the leucocytes counted. From 20 to 40 leucocytes were studied and the average number of organisms then computed.

In serum, normal or otherwise, the meningococcus rapidly undergoes a degenerative change. Even in hanging drop this is shown by the granular appearance, as if disintegration were taking place. In stained preparations it is more evident, many of the cocci failing almost entirely to take the stain, and appearing

more like granules than bacteria. This is particularly true of the meningococci which have been clumped in immune serum. The same phenomenon occurs within the phagocytes, as we should expect, under the influence of intracellular digestion and the strong reducing properties of the protoplasm. Leucocytes containing the organisms, especially after about 20 to 30 minutes, appear filled with granules rather than organisms, and this makes counting frequently very difficult and often impossible. It is therefore advisable to make smears in the phagocytosis experiments after about 10 minutes, so as to avoid as far as possible this rapid disintegration.

TABLE 6.

PHAGOCYTOSIS OF MENINGOCOCCUS.

1.	Normal Human Blood I	13.8
2.	" " " II	11.0
3.	" " " III	18.1
4.	Washed Human Corpuscles	0.1
5.	" " " + heated Serum (60° for 30′)	0
6.	Meningitic Blood I (13th day of disease)	14.8
7.	" " II (7th week of disease)	11.9
8.	" " III (3d week of disease)	12.2
9.	Meningitic washed Corpuscles	0
10.	Normal washed Corpuscles + Normal Serum	13.0
11.	" " " + Meningitic Serum	11.1
12.	Meningitic washed Corpuscles + Normal Serum	9.9
13.	" " " + Meningitic Serum	11.6
14.	Washed Normal Corp. + Men. sensitized with Normal Serum	5.1
15.	" " " + " " " Meningitic Serum	3.9
16.	" " " + " " " Normal Serum	4.6
17.	" " " + " " " Meningitic Serum	5.0
18.	Washed Normal Corpuscles + Cerebrospinal Fluid*	0.1

As the table shows, the meningococci are readily taken up by the leucocytes of normal blood, while washed leucocytes alone or to which heated serum is added are inactive. No essential difference was observed between normal leucocytes and meningitic leucocytes in their power to ingest the organisms. The individual observations vary considerably, but the differences are such as to be within the limits of experimental error. This holds true not only for the defibrinated blood, but in those experiments

*From case of chronic hydrocephalus.

where meningitic serum was added to normal corpuscles, and *vice versa*, and also in the experiments in which meningococci sensitized with normal and meningitic sera were used.

It is quite evident from the above facts that at least two factors are operative in the destructive action of blood upon meningococci, namely, the bactericidal action of serum, and phagocytosis. For an explanation of the former we may resort to Ehrlich's hypothesis and assume the presence of an amboceptor-complement complex present in normal serum, as evidenced by the decreased bactericidal action of heated normal serum, which we may interpret as due to the destruction of the thermolabile complement. In meningitic serum the amboceptor-complement complex is more active through an increase of the amboceptor element, for heating this serum likewise reduces its antibacterial properties by destroying the complement, and hence the two heated sera act alike.

The question naturally arises, why does the meningococcus not grow in heated serum? Compared with normal salt solution, heated serum is distinctly more bactericidal, and this points to the presence of an antibacterial property independent of the amboceptor-complement complex. This is also indicated from the behavior of cerebrospinal fluid, where little or no change is produced by heating; yet it, also, is distinctly more bactericidal than normal salt solution, and, as stated above, acts much like heated serum. The explanation is unknown. It is certainly interesting that a substance like cerebrospinal fluid, which contains practically no cellular elements and extremely small quantities of albuminous substances, should act so differently from salt solution. It may be a question of reaction or the presence of certain salts. This should be investigated further.

The experiments do not indicate any essential change in the opsonin content of the blood during the course of the disease. However, slight differences would not be easy to detect, for the reason that the organisms are ingested with such avidity by the leucocytes in both normal and meningitic blood, and also because the cocci lose their staining property so rapidly inside the cells that the counts are not absolutely trustworthy.

Reference has already been made to Nos. 24 and 25, Table 4, which show that heated serum added to washed corpuscles affords an excellent medium for the meningococcus. Here we have beside the destruction of the complement the destruction of the opsonin of the serum, and consequently phagocytosis is prevented. This experiment, therefore, shows the combined effect of destroying the two chief bactericidal mechanisms of the blood, and results, as we should expect, in the formation of a favorable culture medium.

It is an interesting point that normal cerebrospinal fluid contains no opsonin, as is shown by the fact that no bacteria are taken up when the fluid is added to washed leucocytes. The opsonin which is present in the fluid in the inflammatory state, as evidenced by the phagocytosis in meningeal exudate, arises therefore as a result of the inflammatory process, probably by the exudation of the plasma through the vascular walls. It appears that, in this disease at least, we have merely a change in the distribution of the opsonin to guard against inimical factors rather than any essential change in the total quantity of opsonin in the blood. This may be capable of a more general application.

From the above facts it follows, theoretically at least, that an abundance of opsonin in the meningeal fluid of the spinal canal would favor phagocytic destruction of the infecting meningococci. In the treatment, therefore, of epidemic meningitis it would not be irrational and might be effective to inject into the spinal canal fresh* normal human serum because of its rich opsonin content and also because such serum in addition has a distinct meningococcidal action. Such a fluid theoretically would be more efficacious than the various antiseptics used, which may not only destroy phagocytosis, one of the most important natural protecting mechanisms at work in the disease, but also the meningococcidal complement in the exudate as well. No attempt has been made so far to treat cases in this manner. The practical difficulty of having the serum reach all parts of the meninges involved appears a serious one to overcome, but not more so than in the case of antiseptics injected into the canal.

*It would be necessary to use fresh serum because the opsonins disappear in the course of several days from the serum. In the ice-box it remains active for a much longer time. (See HORTON, *Trans. Chi. Path. Soc.*, 1905, 6, p. 297.)

Recently Wolff,[1] reasoning from certain observations which he made in growing the meningococcus with the B. diphtheriae and in antitoxic serum, suggested the use of diphtheria antitoxin in the treatment of epidemic meningitis. This treatment has been given trial, particularly in New York, by a number of physicians. Their reports are conflicting, some reporting favorably and others not observing any effect on the course of the disease by this treatment.

Wolff states that meningococci sown into diphtheria antitoxin (I assume untreated antitoxic serum, though he does not so state) show a clear serum after 48 hours and also after nine days, devoid or nearly so of organisms—also that three c.c. of antitoxin added to a 24 hour broth culture cause a precipitate in 24 hours and are devoid of organisms after 48 hours. He does not mention control experiments. I have tested the meningococcidal property of normal horse serum and also of untreated antitoxic serum of 600 units strength, kindly furnished me by Dr. A. P. Ohlmacher of Stearns and Company, Detroit, by the same method used in testing the bactericidal property of human sera, and there does not appear to be any essential difference between them in this respect. Horse serum is less favorable for the growth of the meningococcus than human serum. The experiments mentioned by Wolff are absolutely inconclusive so far as demonstrating any specific action of diphtheria antitoxin in meningitis; and he does not show that antitoxic serum acts in any respect different from any other serum.

RÉSUMÉ AND CONCLUSIONS.

Necessarily conclusions deduced from such a limited amount of material as was available for this study must be considered more or less provisional. The following are thus offered:

1. In five cases of epidemic cerebrospinal meningitis the meningococcus (Weichselbaum type) was obtained in every case from the cerebrospinal fluid and in one case from the nose and sputum by culture. In the other four cases Gram-negative diplococci suggestive of either meningococcus or Micrococcus catarrhalis were seen in smears but not recovered in culture.

[1] *American Medicine*, 1905, 9, p. 775.

2. Hemophilous bacilli were found in four of the five cases, being very abundant in two.

3. Agglutination of meningococci by serum of patients with meningitis occurs in dilution of 1:50 or higher.

4. The meningococcus grows in some defibrinated normal bloods but not in others, there being thus an interesting individual variation. In the blood of three meningitic cases it did not grow.

5. Normal human serum is distinctly bactericidal toward meningococci. This property is increased in the sera of meningitic cases and is diminished but not entirely destroyed by heating to 60° for 30 minutes.

6. Cerebrospinal fluid acts in much the same way as heated serum.

7. In the presence of human serum the meningococci are taken up by human leucocytes.

8. The opsonin content of the blood does not appear to be altered during the course of epidemic meningitis.

9. Normal cerebrospinal fluid does not contain opsonin for meningococci.

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